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STUDIES ON THE PHYSIOLOGICAL ACTION OF COLLOIDS. I. THE ACTION OF COLLOIDAL SUBSTANCES ON BLOOD-ELEMENTS AND ANTIBODY CONTENT.

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DURING the past few years a body of evidence has come into being, tending to show that certain infectious conditions may be favourably influenced by the intravenous injection of substances of a colloidal nature. This evidence is chiefly clinical, and valuable results in the most widely varying conditions are attributed to "colloid therapy." In acute febrile diseases, such as typhoid and paratyphoid fever, bacillary dysentery and influenza, Lüdke (1920) considers that an artificial crisis is induced, with subsequent recession of the febrile symptoms. This artificial crisis, it is suggested, is the outcome of a generally increased metabolism, called by Weich and Schrader (1919) an "omnicellular protoplasmic activation," which, presumably, is an acceleration of the "vital activity" of physiologists. But the claims for "colloid therapy" run the whole gamut of pathological conditions—leprosy (Cawston, 1920), pneumonia (Schürer and Eimer, 1923), Hodgkin's disease and leishmaniasis (Samut, 1922), anthrax (Kraus, 1920), and certain cases of mental disorder (Graves, 1920)—and though it is true that attempts by other investigators to repeat these results are not always successful, the evidence in favour of protein therapy is suggestive enough to render the subject worthy of careful study from the experimental point of view.

There is a copious literature upon the subject, briefly referred to at the end of this paper, but it is usually concerned with some particular aspect of the question—the blood-changes brought about by an injection of peptone, the relation between anaphylactic and peptone or colloidal shock, the suppression of shock or a special therapeutic effect. Though there are many general articles ascribing the value of protein therapy to a hyperactivity of normal function, there appears to have been no independent inquiry into the actual nature of the processes concerned, and no systematic investigation of laboratory data. For this reason, the work to be described below has for its purpose

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the determination of a rationale which may explain, in part, the mechanism of response to the injection of colloidal substances. Research has therefore been prosecuted to define precisely the effects of such colloids when injected into normal animals and into animals artificially immunized. The experiments so far undertaken fall into four groups:

(1) The estimation of the limits of effective dosage.
(2) The changes in the blood-elements and temperature of normal animals.
(3) The action of colloids upon the antibody content of artificially-immunized animals.
(4) The non-specific stimulation of antibody production in artificially-immunized animals.

SECTION I.—THE LIMITS OF DOSAGE.

These experiments were carried out to determine the limits within which the injection of colloidal substances might be expected to have a beneficial action, *i. e.* from the point up to which the response is slight and, perhaps, not significant, to the point beyond which the effect of the injection is itself pathological. The colloids used in this series of experiments were:

Collosol ferrum.—The commercial preparation of that name.

Ferric hydroxide sol.—This was made by adding a strong solution of ferric chloride to boiling distilled water (Hatschek, 1920). The free acid was neutralized as far as could be done without precipitation of the ferric hydroxide, but the preparation was still slightly acid when injected.

Collosol argentum.—The commercial preparation of that name.

Metallic silver sol.—Two methods were used in the preparation of this sol—Carey Lea's and Kohlschuetter's (Hatschek, 1920). The first depends on the reduction of silver oxide by dextrin, and, after two experiments, was discarded because of the complicating effect of the dextrin, itself a colloid. The second is made by passing a stream of hydrogen through a filtered suspension of silver oxide, forming an electrolyte-free sol. An attempt was made, in one case, to increase the concentration of this sol by the use of gum arabic (which is physiologically almost inert) as a protective agent, but the sol contained macroscopic particles, and 20 c.c. killed a rabbit in a few minutes. In the later experiments the unmodified Kohlschuetter's sol was used—a clear golden-brown liquid. Both the iron and silver sols used showed active Brownian movement under a dark-ground microscope.

Peptone.—Sterile 5 per cent. and 10 per cent. solutions in distilled water.

Agar and gelatin.—Preparations of various concentrations, which were sols at 37° C., and were made by solution in distilled water in a water-bath.

Methylene blue.—A 10 per cent. solution in distilled water.

Nucleic acid.—A 5 per cent. suspension in distilled water.

The injections were made into normal rabbits, and the doses ranged from 1 c.c. of collosol ferrum to 20 c.c. of a 5 per cent. gelatin sol. The responses to the injections given varied from a slight immediate leucocytosis to acute death in four minutes, but though, for a time, it seemed that the metallic sols were less toxic than the hydrophile colloids, it later became evident that

the difference was primarily one of degree. Each reaction included some of the following changes:

(*a*) A slight immediate polymorphonuclear leucocytosis.
(*b*) A marked immediate polymorphonuclear leucocytosis.
(*c*) An increase in the number of erythrocytes per c.mm.
(*d*) Variations of temperature.
(*e*) A leucopenia followed by a polymorphonuclear leucocytosis.
(*f*) A leucopenia with no recovery.
(*g*) Death after 8 days; death next day; death in 2 hours; death in 4 minutes.
(*h*) No macroscopic *post-mortem* symptoms or congestion of blood-vessels, small hæmorrhages in the intestine, hæmoglobinuria and distended bladder.

The severity of the reaction provoked may be increased or decreased at will by adjusting the volume and concentration of the substances injected. Ferric hydroxide sol is an exception so far as the doses used in these experiments are concerned, since it never induced more than a "mild" shock; possibly a greatly increased dose would be more toxic, but the large volume injected would complicate the effects produced.

Any combination of (*a*), (*b*), (*c*) and (*d*) constitutes a "mild" shock; of (*c*), (*d*) and (*e*) a "severe" shock; a "fatal" shock may include (*c*), (*d*) and (*e*) or (*f*), and in its most acute form is indicated *post mortem* by great congestion of the blood-vessels, hæmorrhages of the intestine, hæmoglobinuria and a distended bladder. The following summary gives the number of experiments performed, and the results obtained in each case:

Silver.—A metallic silver sol induced the whole of the above series of reactions, from a slight leucocytosis to death in four minutes. In the latter case slight hæmoglobinuria was present even in so short a time, but the preparation contained macroscopic particles (see under "Metallic silver sol" above), and the severity of the reaction must be attributed partly to this fact; on the other hand, a 10 c.c. dose (after sedimentation and filtration) caused a "severe" shock, and 20 c.c. of collosol argentum proved fatal in less than two hours, though there were no macroscopic *post-mortem* changes.

(1)	0·5 c.c.	collosol argentum . .	*Nil.*
(2)	1·0 „	„ „ . .	Leucocytosis, increase of red blood-cells.
(3)	1·0 „	„ „ . .	Increase of red blood-cells.
(4)	1·0 „	„ „ . .	Leucocytosis, increase of red blood-cells.
(5)	3·0 „	„ „ . .	Immediate leucocytosis.
(6)	4·0 „	„ „ . .	Leucopenia, leucocytosis
(7)	20·0 „	„ „ . .	Fall of temperature, diarrhœa, death in 2 hours, no *post-mortem* changes.
(8)	0·5 „	Carey Lea sol . .	*Nil.*
(9)	10·0 „	„ „ . .	Leucopenia, leucocytosis, rise of temperature.
(10)	10·0 „	Kohlschuetter's sol . .	Increase of red blood-cells, leucopenia, leucocytosis, rise of temperature, red cell-count normal next day, leucocytosis persisting.
(11)	20·0 „	„ „ . .	Death in 4 minutes, congestion of blood-vessels, slight hæmoglobinuria.

Nucleic acid.—Only a very coarse suspension of nucleic acid could be made without altering its chemical constitution; the injection of one or two

small doses of this suspension resulted in the production of a marked leucocytosis.

(1) 0·75 c.c. 5 per cent. nucleic acid	Immediate leucocytosis.
(2) 1·0 „ 5 „ „	Immediate leucocytosis.

Methylene blue.—After the injection of 10 c.c. of a solution of methylene blue the animal fell into a state of collapse, but had partially recovered in 20 minutes. A leucopenia occurred followed by a leucocytosis, which was still present after 24 hours.

Peptone.—20 c.c. of a 5 per cent. solution of Witte's peptone caused a "severe" shock; the rabbit lost weight and died after 8 days. 20 c.c. of a 10 per cent. solution caused a "fatal" shock; death occurred within 24 hours and the *post-mortem* appearances were again congestion of the blood-vessels, small hæmorrhagic patches in the intestine, hæmoglobinuria and a distended bladder.

(1) 20·0 c.c. 5 per cent. peptone	Rise of temperature, severe leucopenia, death in 8 days, no *post-mortem* changes.
(2) 20·0 „ 10 „ „	Fall of temperature, leucopenia, diarrhœa, death in 24 hours; congestion of blood-vessels, hæmoglobinuria, hæmorrhages and distended bladder.

Gelatin.—The injection of 2·5 c.c. of a 2 per cent. solution was followed by a leucopenia, which was not severe; 10 c.c. of a 5 per cent. solution induced a "severe" shock; 20 c.c. of a 5 per cent. solution caused death in less than 2 hours, with no macroscopic *post-mortem* changes.

(1) 2·5 c.c. 2 per cent. gelatin sol	Leucopenia, rise of temperature.
(2) 5·0 „ 2 „ „	Leucopenia, leucocytosis, rise of temperature.
(3) 10·0 „ 2 „ „	Severe leucopenia, rise of temperature.
(4) 10·0 „ 5 „ „	Severe leucopenia, rise of temperature.
(5) 20·0 „ 5 „ „	Death in 2 hours, no *post-mortem* changes.

Agar.—The injection of 5 c.c. of a 1 per cent. solution was followed by an almost complete disappearance of the polymorphonuclear leucocytes, and five hours later a few nucleated red cells were present in the circulation. 15 c.c. of a 1 per cent. solution caused a reduction of the polymorphonuclear cells (from the normal 50–60 per cent.) to 2 per cent. of a total of 3000 white cells per c.mm.; five hours later the proportion had risen only to 12 per cent.; death occurred within 24 hours and the *post-mortem* appearances were those of acute fatal shock.

(1) 5·0 c.c. 1 per cent. agar	Increase of red blood-cells, severe leucopenia, rise of temperature, leucocytosis, nucleated red cells.
(2) 15·0 „ 1 „ „	Fall of temperature, severe leucopenia, death within 24 hours; congestion of blood-vessels, hæmorrhages of intestine, hæmoglobinuria and distended bladder.

Iron.—This preparation was the least toxic of those employed.

(1) 1·0 c.c. collosol ferrum	*Nil.*
(2) 1·0 „ „	*Nil.*
(3) 2·0 „ „	*Nil.*
(4) 20·0 „ „	Rise of temperature, direct leucocytosis.
(5) 5·0 „ ferric hydroxide sol	Increase of red blood-cells, rise of temperature.

It is apparent from this summary that the same mechanism is concerned in all the reactions to the foreign substances introduced into the circulation in the foregoing experiments; whether the *post-mortem* appearances are also a question of degree is not quite so clear, though it seems highly probable when the effects of gelatin and agar are compared, and the effect of collosol argentum and Kohlschuetter's sol.

SECTION II.—THE CHANGES IN THE BLOOD-ELEMENTS AND TEMPERATURE OF NORMAL ANIMALS.

These experiments were directed towards the production of analogous reactions by modification of the concentrations of the colloidal substances to be injected; it was intended that the reaction should be moderately severe, and that the following should be the component features:

(*a*) The height of the reaction to be over, and a return to normal in progress within six hours.

(*b*) A rise of temperature, returning to normal within six hours.

(*c*) A temporary increase in the number of erythrocytes per c.mm., followed by a temporary fall below normal.

(*d*) A decrease, both relative and absolute, in the number of polymorphonuclear cells, followed by a general leucocytosis.

The colloids used included such diverse preparations as a simple metallic sol, a polysaccharide in solution (dextrin), and a bacterial emulsion. The same volume (5 c.c.) was injected in every case; the effect of a non-toxic crystalloid (5 c.c. of a 1·5 per cent. solution of ferrous sulphate) and the examination of a normal rabbit at hourly intervals served as controls. The results of these experiments are shown in Table I:

TABLE I.—*Red Cell Count* (*in millions*).

	Normal.	0·5% Fe as sol.	1% gelatin.	1% dextrin.	1% peptone.	0·02% Ag. as sol.	0·5% agar.	1000 m. per c.c. typhoid.	0·5 % Fe as ferrous sulphate.
Before injection . .	6·30	5·29	5·92	5·87	6·58	5·56	5·80	6·24	5·64
15 mins. after injection	—	6·24	6·92	5·38	6·63	5·88	6·00	6·89	4·80
1 hour „ „	—	—	—	7·15	—	5·88	5·24	5·63	5·36
2 hours „ „	6·30	6·52	6·16	—	6·27	—	—	—	5·60
3 hours „ „	6·30	—	—	5·35	6·12	5·20	5·60	5·55	—
4 hours „ „	6·50	5·41	6·16	5·17	—	5·12	5·36	—	5·65
5 hours „ „	6·25	4·51	5·52	4·93	5·78	5·12	5·28	4·84	—
24 hours „ „	—	4·80	4·88	5·72	6·40	4·20	5·84	5·78	—

Red cells. Table I. Remarks.—The figures are given in millions of red cells per c.mm. The red-cell count of the normal untreated rabbit is practically constant; that of the rabbit receiving an injection of ferrous sulphate falls immediately, but very soon returns to normal; the initial decrease might be accounted for by the osmotic attraction of water from the tissues—a transitory effect, since the salt rapidly diffuses through the vessel-walls. It is obvious that the action of colloidal substances is not of the same

nature, since the first effect of an injection is, at any rate, a relative increase in the number of erythrocytes. Petersen (1923) shows that the protein content of the lymph is increased after the injection of hæmoglobin, and suggests that the permeability of the vascular wall is altered, so as to permit of the passage of the blood proteins. If this is the case, the osmotic pressure of the blood colloids would be altered, and this, though only 30 mm., is an important factor in the maintenance of blood volume (Bayliss, 1920). Loss of water from the blood to the lymph in this way might result in a temporary concentration of the red cells. On the other hand, if it is true, as Petersen (1923) suggests in the same series of papers, that the increase in permeability is followed within a short time by a change in the reverse direction—a decrease in the permeability of the endothelium—then the foreign colloid present, which is not eliminated for some hours at the earliest (Clark, 1918), might cause an increase in the blood volume, and, consequently, a relative diminution in the number of red cells per c.mm.—the diminution which is shown to occur in the experiments which are the subject of this paper. Actual destruction of the red cells takes place in a severe reaction, as is evident from the hæmoglobinuria, but this is an immediate and not a secondary effect, and it would be difficult to determine to what extent it occurs in the less pronounced reaction. It will be seen from Table I that the red-cell count in the rabbits injected with gelatin and silver had not returned to normal within 24 hours.

TABLE II.—*Temperature Changes.*

	Normal.	Iron sol.	Gelatin.	Dextrin.	Peptone.	Silver.	Agar.	Typhoid.	Ferrous sulphate.
Before injection . .	103·5°	101·8°	100·4°	100·6°	101·3°	101·5°	101·5°	101·4°	103·4°
15 mins. after injection	—	100·6°	101·1°	100·6°	101·2°	102·3°	102·1°	101·0°	102·7°
1 hour „ „	—	—	—	103·2°	—	102·3°	103·2°	102·9°	102·1°
2 hours „ „	103·3°	101·0°	103·6°	—	103·0°	—	—	—	103·3°
3 hours „ „	103·0°	—	—	103·9°	102·7°	102·6°	103·2°	103·2°	—
4 hours „ „	103·5°	100·4°	103·6°	103·1°	—	102·2°	102·7°	—	104·7°
5 hours „ „	103·8°	101·0°	102·0°	100·0°	101·6°	102·5°	102·5°	103·2°	—
24 hours „ „	—	101·7°	101·0°	100·4°	100·8°	—	102·7°	101·7°	103·5°

Temperature. Table II. Remarks.—The temperature (°F.) of the normal untreated rabbit is constant for the period concerned; that of the rabbit receiving an injection of ferrous sulphate shows a slight fall, followed by a slight rise; there was also a slight fall of temperature in the rabbit treated with ferric hydroxide sol, the least toxic of the colloids. In the other six experiments there is an average rise of 1° or 2°F., and the temperature has returned to normal, except in one instance, in 24 hours.

Leucocytes. Table III. Remarks.—The leucocyte count of the normal untreated rabbit shows only slight variations, that of the rabbit receiving an injection of ferrous sulphate shows a direct leucocytosis; the rabbits treated with colloidal substances show a leucopenia followed by a leucocytosis, sometimes persisting for two or three days.

TABLE III.—*White Cell Count.*

	Normal.	Iron sol.	Gelatin.	Dextrin.	Peptone.	Silver.	Agar.	Typhoid.	Ferrous sulphate.
Before injection . .	9,250	13,000	11,750	8,500	5,750	4,750	4,500	7,000	6,500
15 mins. after injection	—	10,750	10,500	4,000	6,250	4,500	3,250	4,500	6,750
1 hour „ „	—	—	—	2,250	—	5,500	7,750	4,000	12,500
2 hours „ „	10,000	21,000	3,500	—	5,250	—	—	—	13,500
3 hours „ „	8,750	—	—	3,500	4,750	4,750	—	1,750	—
4 hours „ „	11,000	20,000	5,500	7,000	—	7,500	4,500	—	14,250
5 hours „ „	10,000	13,000	9,500	10,000	6,750	11,750	6,250	4,750	—
24 hours „ „	—	11,000	26,500	10,000	10,500	—	5,500	16,500	? Food. 21,000
48 hours „ „	—	—	16,750	—	—	7,250	—	12,500	—
72 hours „ „	—	—	—	—	6,000	8,250	—	9,250	11,000

TABLE IV.—*Percentage of Polymorphonuclear Leucocytes.*

	Normal.	Iron sol.	Gelatin.	Dextrin.	Peptone.	Silver.	Agar.	Typhoid.	Ferrous sulphate.
Before injection . .	—	40·5	37·7	42·1	66·6	19·1	35·6	38·6	40·9
15 mins. after injection .	—	? 10·0	35·0	19·6	54·4	32·0	—	35·3	17·0
1 hour „ „ .	—	—	—	9·8	—	48·4	37·9	21·9	29·8
2 hours „ „ .	—	60·0	11·0	—	58·2	—	—	—	37·0
3 hours „ „ .	—	—	—	36·1	53·6	36·3	—	6·0	—
4 hours „ „ .	—	59·5	? 66·5	82·5	—	50·0	—	—	78·4
5 hours „ „ .	—	—	46·5	93·8	51·4	50·0	—	12·5	—
24 hours „ „ .	—	30·0	56·3	55·8	42·7	—	—	64·0	—
48 hours „ „ .	—	—	22·4	—	—	—	—	55·4	—
72 hours „ „ .	—	—	—	—	—	15·0	—	60·0	—

Differential leucocyte counts. Table IV. Remarks.—This table shows that the polymorphonuclear leucocytes are chiefly concerned in the leucopenia and leucocytosis.

Effect of a Second Injection into the Same Animal.

A further experiment was carried out on four of the rabbits used in the above series, to determine whether the effect of a second injection of the same substance into the same rabbit would correspond with that of the first. The results are very similar, but suggest, in the case of typhoid, that toleration may be established in time.

Effect of a Second Injection. Table V. Remarks.

Typhoid.—This was the third injection of 5000 million typhoid bacilli given within 17 days. The leucocytic changes are less marked, but they follow the same sequence as those of the first injection. The other changes are similar.

Dextrin.—Interval between experiments, 6 weeks. The changes here correspond with those of the first experiment.

TABLE V.—*Red Cell Count. Temperature. White Cell Count.*

	Typhoid.	Dextrin.	Silver.	Ferrous sulphate.
RED CELLS.				
Before injection	5·90	6·33	5·16	5·85
15 mins. after injection	5·82	7·12	5·24	5·80
1 hour „ „	6·22	7·08	5·13	5·67
3 hours „ „	5·34	7·50	4·63	6·00
5 hours „ „	5·04	6·76	4·80	6·10
24 hours „ „	5·21	6·73	4·95	—
TEMPERATURE.				
Before injection	101·7°	102·3°	102·0°	101·8°
15 mins. after injection	102·1°	103·1°	102·0°	101·4°
1 hour „ „	104·4°	104·5°	103·8°	103·7°
3 hours „ „	105·1°	104·0°	103·8°	102·9°
5 hours „ „	104·1°	103·2°	103·8°	103·2°
24 hours „ „	101·9°	102·4°	101·5°	—
WHITE CELLS.				
Before injection	6,500	10,750	5,500	8,000
15 mins. after injection	4,250	5,750	6,000	8,000
1 hour „ „	2,500	4,250	5,500	12,000
3 hours „ „	4,000	2,250	6,500	12,500
5 hours „ „	5,500	11,500	6,750	11,000
24 hours „ „	9,500	10,750	10,250	—

Silver sol.—Interval, 6 weeks.

Ferrous sulphate.—Interval, 2 weeks. The data in both these cases agree with those of the first experiment.

It will be seen from the tables given above that the reactions provoked by different colloids are comparable. The dose of peptone was too small to cause a decided leucopenia. The record of the experiment with agar is not in conformity, probably owing to technical error, but the result of the work in Section I with both peptone and agar show that the sequence of changes in the white cells follows that of the other experiments recorded here. The duration of the leucocytosis appears to depend on the severity of the reaction. Gelatin and typhoid emulsion caused the most severe reactions, and, in these cases, the leucocytosis persisted for two or three days. A consideration of these tables suggests that the basis of the defensive mechanism of the animal body is inherent, and that the response elicited is not primarily specific to the stimulus by which it is evoked.

SECTION III.—THE ACTION OF COLLOIDS UPON THE ANTIBODY CONTENT OF ARTIFICIALLY IMMUNIZED ANIMALS.

In view of the beneficial results obtained in certain infectious processes, and attributed to the intravenous administration of colloids, the possibility suggested itself that these substances might have a direct action upon the antibodies concerned. It may be stated at the outset that all the experiments on this point were negative. Rabbits were used, in which the agglutinins had reached a maximum and then fallen to a constant level; the doses given were of the same volume and concentration as those used in the previous series, but

the only change determined was that the agglutinating power of the immune serum was temporarily decreased. Where the differences of the serum dilutions were within narrow limits, there were slight fluctuations of the titre of the serum from day to day; where the difference in dilutions was 100 per cent. the variations were inappreciable. The results of three experiments are given as examples in Table VI:

TABLE VI.—*Typhoid Agglutinins.*

		Iron sol.					Gelatin.					Silver sol.		
		1/800.	1/1600.	1/3200.			1/800.	1/1200.	1/1600.			1/100.	1/200.	1/400.
Stationary titre before injection,	March 17	++	+	−	.	May 1	+++	++	tr.	.	May 13	+	+	+
After one injection of colloid	„ 18	++	−	−	.	„ 2	++	tr.	−	.	„ 14	++	++	+
	„ 19	++	tr.	−	.	„ 3	+++	++	tr.	.	„ 15	+	tr.	−
	„ 20	++	−	−	.	„ 5	++	−	−	.	„ 16	++	+	tr.
	„ 22	++	−	−	.	„ 6	++	−	−	.	„ 17	+	+	−
	„ 24	++	−	−										

SECTION IV.—THE EFFECT ON ANTIBODY PRODUCTION OF A COMBINATION OF COLLOID WITH A BACTERIAL ANTIGEN.

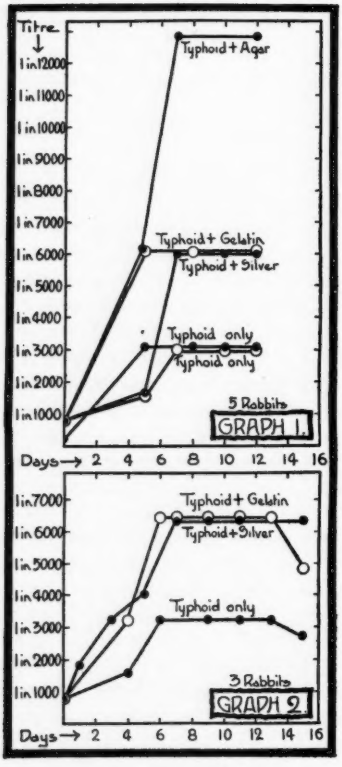
The variation of the quantity of bacterial antigen injected produces, within limits, a parallel variation in the development of agglutinins; if the amount of antibody finally present were a function of the quantity of antigen injected, it should be possible to increase the production of agglutinins up to the point at which the dose of antigen provokes a fatal "colloidal shock." This cannot be done. It may be that the production of antibody is related in some way to the primary disturbance of normal conditions consequent upon the injection. In this case there should be an *optimum* reaction, and it should be possible to increase the development of agglutinins following a small dose of antigen by increasing the "shock" of the injection. So far as can be determined by the experiments recorded above, the injection of a bacterial emulsion gives rise to the same immediate sequence of changes in the body as the injection of an inorganic or organic non-protein colloid. Theoretically, therefore, the injection of a small quantity of a bacterial emulsion combined with a small dose of some substance producing the same kind of effect upon the body should induce a shock equivalent to that consequent upon a larger injection of either agent. If the production of antibody is in part dependent on the degree of primary shock, then the injection of another colloid together with the antigen should intensify the reaction excited, and the result should be a greater production of antibody than that consequent upon the use of antigen alone. The following experiments were designed to yield information on this point:

(*a*) Seven normal rabbits were given an intravenous injection of 1000 million killed typhoid bacilli. Three hours later intravenous injections (2 c.c.) of gelatin, silver, iron, dextrin and agar were given to five of these rabbits, on the assumption that the second reaction induced would stimulate further the processes initiated by the first. Similar colloidal injections were made on the

second, third and fifth days. On the eighth day the agglutination titres of the serum of these rabbits was as shown in Table VII.

TABLE VII.

	Agglutination titre.			
	1/800.	1/400.	1/1600.	1/3200.
Typhoid only	++	+	+	−
Typhoid only	++	++	+	−
Typhoid + gelatin	++	++	++	±
Typhoid + silver	++	++	++	+
Typhoid + iron	++	++	++	++
Typhoid + dextrin	++	++	+	−
Typhoid + agar	++	++	++	++



(*b*) Six rabbits were taken which had received three intravenous injections of typhoid. The agglutination titre had risen to the same height and fallen to the same level in the same time. In three rabbits this stationary titre was 1/1600, and in the other three 1/800. All six were given an intravenous injection of 300 million killed typhoid bacilli; two were given at the same time 5 c.c. of a gelatin sol, and two, 5 c.c. of a silver sol. The remaining two served as controls. Graph 2 shows the maximum titre reached, and the time taken to reach it. Only the curves of the rabbits with a stationary titre of 1/800 are given, for the sake of clearness, but a similar result was obtained in the case of the other three rabbits.

(*c*) Another series of five rabbits, each having previously received seven intraperitoneal injections of 100 million killed typhoid bacilli, and each with a titre which had risen to 1/3200, and fallen to 1/800 (except No. 40, which rose to 1/800 and fell to 1/200), were given 300 million killed typhoid bacilli intravenously. One received an accompanying injection of colloidal silver, one of gelatin and one of agar, while the other two served as controls. The agglutination curves are shown in Graph 1.

(*d*) In some of the experiments the changes in the agglutinating power of the serum were observed at early periods after injection. It is interesting to note, in the examples given in

Table VIII, that the decrease of agglutinating power, and its return to "normal," correspond fairly closely in time with the cycle of changes in the defensive reaction described in Section II.

TABLE VIII.—*Typhoid Agglutinins.*

	Typhoid only.			Typhoid + gelatin.		
	1/100.	1/200.	1/400.	1/200.	1/400.	1/800.
Stationary titre before injection .	++	+	−	++	+	−
1 hour after injection of typhoid .	++	−	−	++	tr.	−
3 hours " " " .	++	tr.	−	++	−	−
5 hours " " " .	++	tr.	−	+	−	−
1 day " " " .	++	tr.	−	tr.	−	−
2 days " " " .	++	+	tr.	tr.	tr.	−
3 days " " " .	+++	++	+	++	+	−
4 days " " " .	+++	+++	++	++	++	++

Note on agglutination technique.—The agglutination tests in these experiments were carried out with standard formalized emulsions of *B. typhosus*, made by the rapid suspension in normal saline of 24-hour agar cultures, and dilution to a standard opacity. These emulsions were made in quantities of 750 c.c., and used throughout the course of a series of experiments, so that the conditions of the tests might be uniform. The agglutinating properties of the emulsion in use were examined at intervals by means of a stock immune serum. All vaccines used for injection were made by dilution of the same emulsion.

GENERAL DISCUSSION OF EXPERIMENTS IN THE LIGHT OF PREVIOUSLY RECORDED DATA.

The information furnished by the experiments recorded in this paper shows that the simultaneous injection of colloidal substances with an antigen has a definite effect upon the amount of antibody produced, and upon the rate at which this production rises to a maximum. In each case the colloid caused a greater development of agglutinin at a greater rate, so that it would appear that the action of a colloidal injection, concurrent with that of the antigen, is to shorten the time taken to arrive at a maximum. This bears some analogy to the production of an artificial crisis in typhoid fever (Lüdke, 1920).

The results of an injection which produces fatal colloidal shock are not, to superficial observation, distinguishable from the phenomena of anaphylaxis. Dale and Kellaway (1922), however, show definitely that, in the case of anaphylatoxins, the specific stricture of the bronchial musculature of the guinea-pig is absent, and, after exhaustive experiments on the subject, they draw the conclusion that the injury is mainly referable to changes in the blood and endothelium, although, at the same time, there are perplexing relations between the two types of shock. Colloidal shock is similar to the latter type, since here also the blood and vascular endothelium seem to be the seat of primary injury. Hanzlik and Karsner (1919, 1920), in a long series of experiments on the action of substances, chiefly of a colloidal nature, also differentiate between anyphylactic and "anaphylactoid" symptoms in shock, while Manwaring (1923) shows that peptone shock occurs in the dog after the

liver has been excluded from the circulation. Brodin and Richet (1921) distinguish between the changes occurring in anaphylactic and peptone shock; they say that inversion of the leucocyte formula occurs, and nucleated red cells are found in anaphylactic, but not in peptone shock; both these changes, however, took place in the reactions described here.

There is general agreement, clinically, as to the result following upon an injection of protein—chill, rise of temperature 4° to 5° F., leucopenia, leucocytosis with neutrophil myelocytes and normoblasts (Petersen, 1921; Gow, 1920; Adelsberger and Rosenberg, 1923), and these are in accord with the laboratory results of Section II. Experimentally, Seeliger and Gorke's (1921) record of the changes in the blood-cells following peptone injections correspond with those in this paper, and Dean and Webb (1924), in some recent experiments, established that an increase in the number of erythrocytes per c.mm. occurs very soon after the injection of serum into a normal animal.

The laboratory evidence is contradictory upon the subject of non-specific stimulation of antibodies. Herrmann (1918) says that there is a rise of streptococcal and meningococcal opsonins and agglutinins following upon the injection of a foreign protein, but there is no rise in typhoid agglutinins. Fürst (1920) showed that a small injection of methylene blue four days later than the antigen would increase the production of agglutinins. McIntosh and Neave Kingsbury (1924) have been unable to repeat the findings of Walbum (1921) as to the chemical stimulation of antibody production. Khanolkar (1924) showed that previous treatment with non-specific substances may or may not influence the production of agglutinins for *B. Gaertner*. In his experiments the test doses were given some weeks after the non-specific injections; his negative results, which occur chiefly with non-bacterial proteins, might be explained by the findings of this paper on the ground that the temporary reaction following upon the injection of a foreign protein merely reinforces an already active and recently stimulated defensive mechanism, and therefore would have no effect after so long an interval.

The foregoing brief references to current literature indicate that no definite conclusion on this subject is yet available, but the findings in this paper show that the results of the injection of a colloid (which is the ultimate basis of non-specific therapy) can be predicted with a fair degree of accuracy, provided that the particular range of effect produced by the substance employed has been determined experimentally. It seems clear, too, that colloids may beneficially accelerate the physiological processes of defence, provided they are used under the right conditions. It might also be possible, though an active production of antibody does not necessarily mean an equally active immunity, to determine whether this "reinforcement" of normal function can be correlated with a greater resistance to actual infection, particularly in chronic conditions, and an attempt will be made, in the next series of experiments, to show how such conditions may be affected by colloidal injections.

SUMMARY.

(1) It is shown that the reactions caused by all the colloids used are of the same order, but that the degree of the reaction varies with the nature of the colloid and with the amount injected.

(2) A more detailed examination of the changes concerned shows that the factors of the primary response are probably the same in all the experiments.

(3) No evidence was obtained that the injection of a colloid can increase the agglutinin content in an animal with a stationary titre.

(4) Details of experiments are given which suggest that the antibody response to antigen is reinforced by the simultaneous injection of a colloidal substance.

I should like to record here that I owe a great deal to Dr. Ledingham's unfailing interest and help.

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A ROTARY NOMOGRAM FOR USE IN THE CALCULATION OF DIABETIC AND OTHER DIETS.

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THE following is an extension of, and, it is hoped, an improvement on the series of nomographic charts previously suggested by the author (1923).

Theory of the rotary nomogram.—Let it be required to determine the calory value of a diet of P grams of protein, C grams of carbohydrate, and F grams of fat. Each gram of food substance must be multiplied by its appropriate factor (say, p, c and f respectively), in order to determine its calory value. The products must then be added together in order to give the total calory value Q. We thus have an expression of the form—

$$Q = pP + cC + fF.$$

In practice, p and c are approximately equal, each being about 4·1, and f is 9·3. Hence we may group the protein and carbohydrate together and write the equation as—

$$Q = p\ (P + C) + fF.$$

This may easily be adapted to nomographic representation in either of the two standard ways:

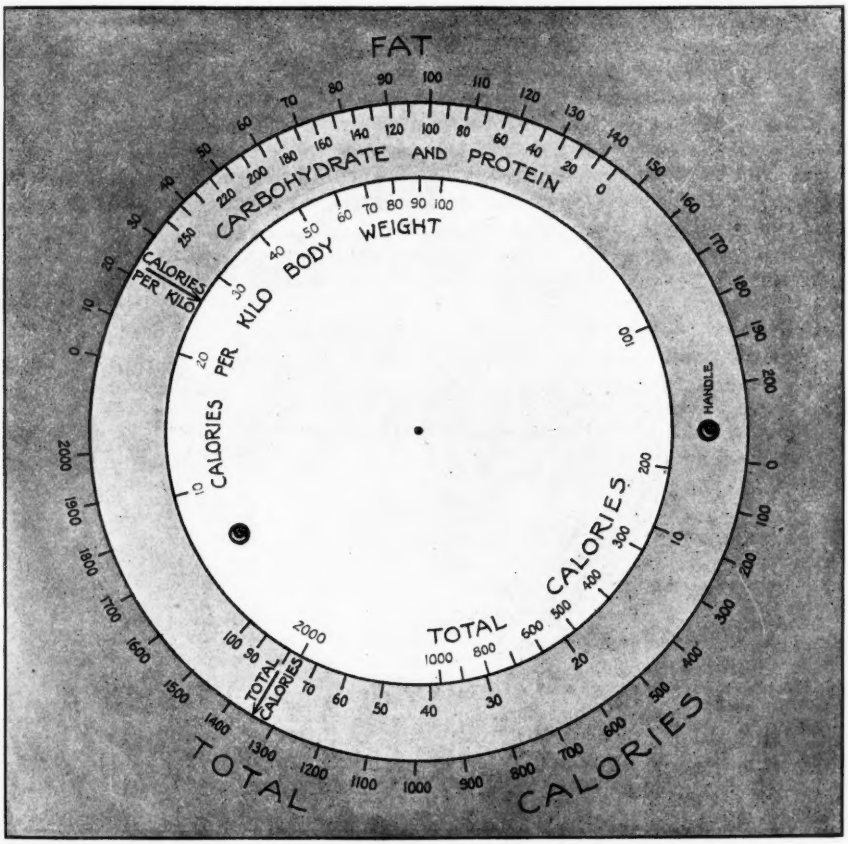
1. Three parallel equidistant columns, divided into unequal scales, such that the $P + C$ scale is $2p$ times the Q unit, and the F scale $2f$ times the Q unit, the latter being, of course, arbitrary.

2. Three parallel non-equidistant columns separated in the ratio $f:p$, the food columns being divided into equal scales and the calory column with its scale unit $1/(f + p)$ of that of the other columns.

Both of these methods, however, require the use of a ruler or straight-edge in order to secure the required collinearity. It occurred to the author that a neater and more ready-to-hand form could be made by arranging the various columns around the circumference of a circle. The equidistant parallel column method can very conveniently be adopted for this purpose, for it is readily seen that the three columns can be made to lie on the circumference of the *same* circle. Using astronomical language, the two food columns will be in conjunction, and the total calories in opposition. The ratio of the scale units of the two former columns would be $p:f$. For the rotation of the columns a handle may be placed in quadrature on the right-hand side. It should be noted that in the corresponding linear nomograms the scales all run the same way—either upwards or downwards. If we convert these to a rotary nomogram the reading of the two scales in conjunction must be in opposite ways, since they represent the outer columns of the original linear nomogram.

Again, let it be required to find the calories per kilo body-weight, K, of the diet. We therefore require the value of the quotient $Q/K = R$, say. This is the same as—

$$\text{Log. } Q = \text{log. } K + \text{log. } R.$$



The equation, written in this form, is obviously of the same type as the above, except that logarithms replace the natural numbers. We must therefore divide up the columns logarithmically, and also make the scales in the ratio of equality. We can similarly arrange it around the circumference of a circle. In this case, however, all the scales read in cyclic order, as we are really dealing with a subtraction (log. Q — log. K) and not a sum.

For the sake of convenience this second circle may be concentric with the first, and set within it. In this the two columns in conjunction are the total

calories, and the body-weight, with their scales reading the same way.* The column in opposition gives the result required, viz. the calories per kilo. The handle for its rotation may likewise be placed in quadrature, but on the left-hand side, *i.e.* in opposition to the other handle. To secure further convenience, the two columns in conjunction may themselves be in opposition to the corresponding two of the first nomogram.

To use the rotary nomogram in practice.—Rotate the larger sized circle by the handle provided on the right-hand side until the prescribed number of combined grams of protein and carbohydrate is opposite the number of grams of fat. The arrow at the bottom of the same circle points to the total number of calories yielded by the above. A similar procedure must be gone through for the determination of the calories per kilo. For this purpose rotate the inner card by the handle on the left-hand side until the total number of calories is opposite the body-weight of the patient. The arrow at the top of the figure will show the total calories per kilo required.

The nomogram may also be used in a converse manner. That is, corresponding to any required number of total calories, we may at once obtain a great variety (theoretically infinite) of possible combinations of protein, carbohydrate, and fat, yielding the same amount of heat.

The diagram shows the nomogram set for a diet of 100 grams of fat and 100 grams of mixed protein and carbohydrate. The arrow at the bottom registers 1340 as the total calories yielded by this diet. Lastly, the inner card is shown set at 1340 total calories for a 50-kilogram patient. The top arrow shows the final result of 26·8 calories per kilo.

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A CONTRIBUTION TO THE ÆTIOLOGY OF EXPERIMENTAL KERATOMALACIA.

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Received for publication November 27th, 1924.

IN 1913 Osborne and Mendel discovered that rats fed on diets deficient in vitamin A develop an inflammatory condition of the eyes, the disease being similar both clinically and pathologically to the keratomalacia originally described by Blessig (1866) as occurring among the poverty-stricken population of St. Petersburg.

* The title of the "body-weight" scale has been omitted from the diagram by mistake.

Since 1913 many observers have confirmed the relationship between keratomalacia and a deficiency of vitamin A in the diet, for the disease has now been produced experimentally not only in rats, but in dogs, rabbits, guinea-pigs, mice, hens, ducks and pigeons. The reason, however, why an absence of vitamin A should lead to keratomalacia is still unknown, though the suggestion has been made that possibly some change may occur in the secretion of the paraocular glands. Clinically the onset of keratomalacia is associated with increased lacrymation, which lasts for one or two days, after which the secretion becomes reduced in amount and sero-sanguineous in character. Histologically, the conjunctival epithelium undergoes hyalinization during the period of increased lacrymation, while examination of the paraocular glands in rats has so far failed to demonstrate any characteristic lesion in the lacrymal, though degenerative changes have been noted in the Harderian gland. Although well developed in rodents, the Harderian gland is

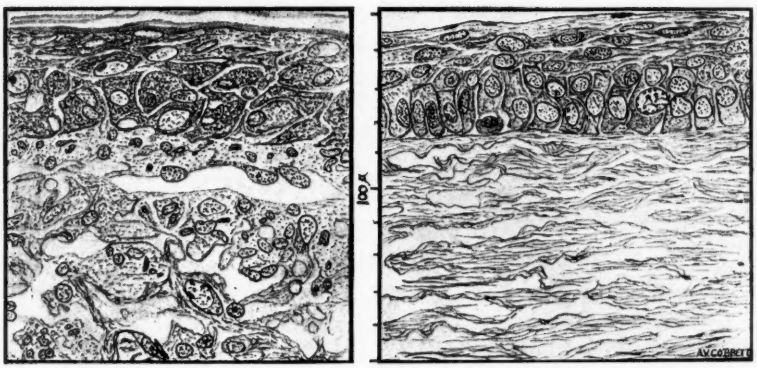

FIG. 1.—The cornea from a normal rat (right), and from a rat fed on a diet deficient in vitamin A (left), showing the hyalinization of the surface epithelium. Stained van Gieson and iron-hæmatoxylin.

only rudimentary in man, in whom keratomalacia is by no means uncommon; it therefore seems unlikely on *a priori* grounds that a change in the secretion of this gland alone can be sufficient to set up the train of events which leads to keratomalacia.

In the present paper it is proposed to bring forward experimental data which lend some support to the suggestion that absence of vitamin A in the diet leads to a change in the secretion of the paraocular glands.

The rats used in these experiments were all bred from laboratory stock, and when 40–50 gm. in weight were placed on the following diet:

Casein	14 gm.
Starch	77 „
Marmite	2 „
Inorganic salt mixture . .	7 „

On this food young rats fail to gain in weight after the first few days, and in at least 80 per cent. of cases develop keratomalacia in from 35 to 50 days.

(A) ATTEMPTS TO PRODUCE KERATOMALACIA BY THE INOCULATION OF BACTERIA INTO THE CONJUNCTIVAL SAC OF THE RAT.

One of the first questions which naturally arises in connection with the study of keratomalacia is the *rôle* of bacteria in its causation.

The types of bacteria present in the conjunctival sac of the healthy rat and that of the rat with keratomalacia have been studied in considerable detail by Stephenson and Clark (1920). There is not only no specific organism present in keratomalacia, but no significant difference in the types of organism found in the conjunctival sac of the healthy and keratomalacic animal. Notwithstanding the similarity in type, it appeared possible that in keratomalacia the organisms might have an increased virulence.

Experiment 1.—Material aspirated from the conjunctival sacs of 3 rats suffering from advanced keratomalacia was inoculated directly into the conjunctival sacs of 12 normally fed rats. No case of keratomalacia developed.

Experiment 2.—Material derived from the same source as in Experiment 1 was inoculated directly into the right conjunctival sacs of 12 young rats which had been fed for 25 days on a diet lacking vitamin A. The dates of onset of keratomalacia are shown in Chart 1, in comparison with the dates for 12 uninoculated rats fed on the same deficient diet.

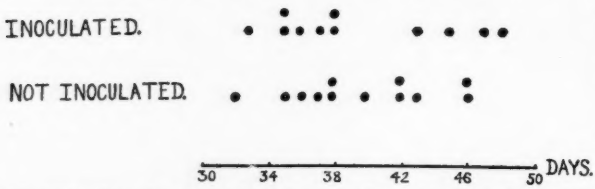

CHART 1.—Showing the dates of onset of keratomalacia in control rats on a diet deficient in vitamin A, and in similarly fed rats inoculated with bacteria in the right conjunctival sac.

It will be seen that the inoculation of bacteria into the conjunctival sacs of deficiently fed rats did not hasten the onset of keratomalacia, while the disease, when it occurred, was not limited to, or more severe in, the right eye.

(B) THE *RÔLE* OF LYSOZYME IN THE PREVENTION OF KERATOMALACIA.

The work of Fleming (1922) and Fleming and Allison (1922) has shown that human tears have a powerful lytic action on certain bacteria. The substance which causes the lysis has been termed lysozyme: its action can be readily demonstrated on a coccus—*Micrococcus lysodeikticus*. Unfortunately the tears of the rat and guinea-pig have little or no lytic action on *M. lysodeikticus*, though both the tears and blood-serum of rabbits possess this power. It was therefore of interest to determine whether there was any change in the lysozyme content of the tears and serum of rabbits fed on diets lacking in vitamin A. Three rabbits were placed on a diet deficient in vitamin A, similar to that given to the rats except for the addition of olive oil. The lytic titre of the tears and blood-serum was determined at intervals, 3 normally fed rabbits being used as controls. The technique was similar to that described by Allison (1924), while the lytic action was tested against *M. lysodeikticus*, for cultures of which I am greatly indebted to Prof. Fleming. The results are shown in Chart 2.

It will be seen that at the onset of keratomalacia there is a definite decrease

in the lysozyme content of the tears, this decrease becoming more marked after 2 to 3 days. There is little or no fall in the lysozyme content of the serum.

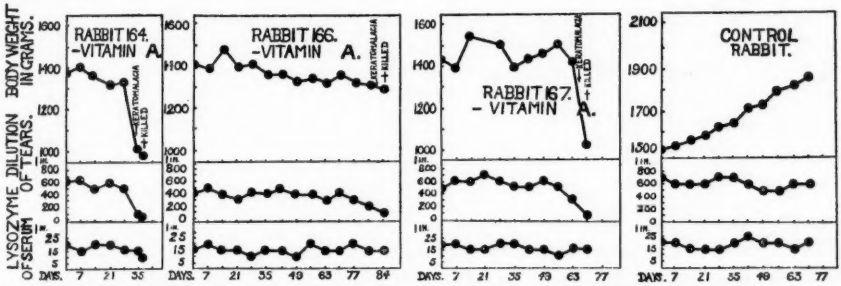

CHART 2.—The body-weight of a control rabbit and of rabbits fed on a diet deficient in vitamin A, and the highest dilutions of tears and of blood-serum having a lytic action on *M. lysodeikticus*.

(c) ATTEMPTS TO PREVENT THE ONSET OF KERATOMALACIA.

Experiment 1.—Twenty young rats were fed on a diet lacking vitamin A for 25 days. The rats were then divided into two batches of 10 each. The first received no treatment. Nine developed keratomalacia; 1 died from broncho-pneumonia without symptoms of eye trouble on the thirty-fourth day of the experiment. The other batch had their conjunctival sacs carefully washed out night and morning with undiluted human tears. One case of keratomalacia developed on the thirty-fourth day of the experiment. All the other animals eventually died with broncho-pneumonia without any signs of eye involvement. The average length of time on the diet till death was 44 days for the control, 48 days for the treated rats. Histological examination showed that in the control rats hyalinization and necrosis of the conjunctiva were much more advanced than in those whose conjunctivæ had been washed with tears. Bathing with tears did not appear to bring about any material change in the character of the bacterial flora of the conjunctival sac.

Experiment 2.—A similar experiment to the above, except that the tears were heated for 30 minutes at 70° C.; 2 of the 10 treated rats developed keratomalacia, while all the controls suffered from the disease. Heating tears to 70° C. decreases the amount of lysozyme in the tears, but traces can still be detected.

Experiment 3.—Locke's solution was used in place of tears. Four out of 10 treated rats developed keratomalacia.

Experiment 4.—Physiological saline solution was used in place of Locke's solution. Here 8 out of 10 treated rats developed keratomalacia in from 34 to 49 days.

(d) ATTEMPTS TO PRODUCE KERATOMALACIA MORE RAPIDLY BY STIMULATION OF THE PARAOCULAR GLANDS.

Experiment 1.—Six normal rats were inoculated subcutaneously with 0·01 gm. pilocarpine hydrochloride twice daily for 5 days. There was increased

lacrymation immediately after the injections, but on their cessation no further symptoms of keratomalacia developed.

Experiment 2.—Six young rats fed on a diet deficient in vitamin A for 24 days were given similar injections of pilocarpine hydrochloride for a like number of days. At first there were the usual symptoms of increased lacrymation following on the injections, but after 4 days the secretion was reduced in amount and became sero-sanguineous in character. The same day the conjunctivæ were inflamed, while 2 or 3 days later there was noticeable opacity of the cornea.

DISCUSSION.

As the result of these experiments some support is forthcoming for the suggestion that the essential cause of keratomalacia lies in a change in the character of the secretion of the paraocular glands. On the one hand the bacteria commonly found in keratomalacia cannot by themselves initiate the condition; the soil must be prepared. On the other hand, the fact that the onset of keratomalacia may either be precipitated by excessive stimulation of the paraocular glands, or prevented by bathing out the conjunctival sac with tears would seem to indicate that it is the secretion of tears which plays the all-important part in the causation of the disease. The secretion of the paraocular glands might conceivably prevent the bacterial invasion of the conjunctiva in three ways:—(1) By washing out bacteria from the conjunctival sac; (2) by its bacteriolytic action; (3) by maintaining the nutrition of the conjunctiva. By their mechanical action the tears prevent bacteria from coming into direct contact with the conjunctival epithelium. There is probably, however, something more than a mere mechanical action, since Locke's solution is not as efficient as tears in preventing keratomalacia, while physiological saline solution is of no value as a preventative; it may in fact be harmful to the conjunctival epithelium. The hyalinization of the epithelium would appear to be directly related to a reduction and an abnormal constitution of the lacrymal secretion. There is direct evidence that as the result of the deficiency of vitamin A the lacrymal secretion is deficient in lysozyme, but in addition it is possible that some alteration in the inorganic constituents of the secretion may occur—a suggestion which is supported by the experiments of Yudkin (1924), who finds that the absence of both phosphorus and vitamin A in the diet leads to a more rapid onset of keratomalacia than the absence of vitamin A alone.

The exact *rôle* played by lysozyme in preventing keratomalacia is difficult to assess, though it is noteworthy that unheated tears are very effective in preventing keratomalacia, tears heated to 70° C. are a little less efficient, while Locke's solution is even more unsatisfactory.

CONCLUSIONS.

Experimental evidence is brought forward in favour of the hypothesis that the absence of vitamin A in the diet causes a change in the secretion of the paraocular glands, as a result of which hyalinization of the conjunctiva occurs, accompanied by bacterial invasion of the tissues.

The lysozyme content of the tears of rabbits is decreased as the result of a diet deficient in vitamin A.

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THE SEXUAL GLANDS AND METABOLISM. I. INFLUENCE OF CASTRATION ON NITROGEN AND GASEOUS METABOLISM.

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HISTORICAL.*

Obesity after Castration.

IT is a well-established fact that after castration in man the amount of fat in the body sometimes increases so that a pronounced degree of obesity develops. The climacteric in women has the same effect upon fat deposition as has the removal of the ovaries. However, this obesity found after castration and the climacteric is not observed in all cases. Glaeveke (1889) found the following changes in the weight of 40 women after castration: considerable increase in 57·5 per cent. of them (average increase 17 lb. and a maximum of 40 lb.), small increase in 35 per cent. (maximum increase of 10 lb.), and no change in 7·5 per cent. Changes in weight after castration of 116 women were also investigated by Pfister (1898), with the following results: increase in weight in 52 per cent., no change in 30 per cent., decrease in weight in 18 per cent. Tilt recorded the following changes in weight of 282 women after the climacteric: increase in 43 per cent., no change in 25 per cent., and decrease in 32 per cent.

Some species of castrated animals, *e.g.* birds, develop obesity so regularly after the removal of the sexual glands that this operation is employed

* Elsewhere (Korenchevsky, 1923) I have summarized and critically reviewed all investigations concerning calcium and phosphorus metabolism after castration.

commercially for fattening animals for food. Heymans (1921) compared the amount of peri-abdominal fat in 2 cocks, and 2 capons of about the same weight, and found that the average weight of fat was 51 gm. in the cocks and 178 gm. in the capons. In my experience with laboratory animals (dogs, rabbits, rats), I have found a pronounced increase in the fat deposition in the body on autopsy in about 70 per cent. of the animals.

This frequent accumulation of fat in the body after removal of the sexual glands is alone sufficient to suggest a change in the metabolism of a castrated animal. But at the same time I want to emphasize that castration in man as well as in animals does not always produce obesity. Mottram and Cramer (1923) were unable to observe any obesity in mice during 18 weeks after castration. Therefore it is of great interest to study the metabolism in "fat" as well as in "thin" castrated animals.

In previous investigations very little attention has been paid to this fact.

Gaseous Metabolism after Castration.

Animals.—Poppel (1897) performed experiments on 6 rabbits. Unfortunately the amount of food eaten as well as of water drunk varied. Therefore the results obtained from his metabolism experiments cannot be regarded as conclusive. The changes found in the gaseous metabolism were not constant, but in most cases there was a decrease in CO_2 expired.

Loewi and Richter (1899) were the first to perform any accurate experiments, using 1 bitch and 1 dog. After removal of the sexual glands they found a decrease from normal in the gaseous metabolism of 10 to 20 per cent. This effect of castration was constant during the whole experiment, which lasted for about 2 years (after castration).

Lüthje (1902) compared the gaseous metabolism of 2 castrated dogs with that of 2 control animals from the same litter, and could find no difference within $1\frac{1}{2}$ to 2 years after castration. His experiments were performed with faultless technique. At the end of the experiment the dogs were killed and the fat content of their bodies investigated. In one of the two castrated dogs fat was present in a larger amount than in the other, but Lüthje explains this by the larger build of the former animal.

A diminution in the gaseous metabolism of 17 to 30 per cent. after castration was found by De Vita (1913) in 2 puppies (castrated very soon after birth), by Murlin and Bailey (1917) in 2 bitches (till the 35th day after castration), by Kojima (1917) in 6 rats (till the 48th day after castration), by Heymans (1921) in 5 capons (till the 7th year after castration), and by Tsubura (1923) in 14 rabbits (2–182 days after the removal of the sexual glands).

Bertschi (1920), on the contrary, was unable to find any change in the CO_2 and H_2O exhaled by rabbits (2 males and 2 females) during 3 weeks following castration. Bertschi's experiments therefore give the same negative results as those of Lüthje mentioned above. Cramer and Marshall carried out observations on ovariotomized rats, and failed to find any evidence of a diminution in respiratory exchange, as of a tendency to adiposity; no details are given at all.

Human beings.—The gaseous metabolism has been studied by Zuntz (1904, 1908, 1912) in 6 women successfully. A diminution in the gaseous metabolism was found in 3 women only, no definite changes being observed in the remaining 3. He explains this difference by the supposition that in the latter cases the ovaries were much changed pathologically when removed, and therefore their absence did not cause any change in the metabolism. Loewy and Kaminer (1916) also observed a decrease in the oxygen consumption of a soldier completely castrated by a bullet. When testicular opotherapy was applied, the oxygen consumption increased. Kraul and Halter (1923) produced a temporary degeneration of ovarian tissue by Roentgen rays in 5 patients. A few days after radiation the metabolism was found to have increased. According to the author's explanation this was due to the absorption of destroyed ovarian tissue. Three months after radiation all the patients weighed 2–4 kilos more, and their metabolism was reduced as much as 17 to 30 per cent.

Basal metabolism was also investigated by Zuntz (1912) in 2 eunuchoids, in whom no change from the normal was observed, and by Tierney (1920) in a patient suffering from ovarian insufficiency. In the latter case the gaseous metabolism was 18 per cent. below the normal average. Harvier and Van Bogaert (1924) found a considerable decrease in the basal metabolism of 1 out of 4 patients suffering from testicular insufficiency.

Summarizing the results obtained by these many workers, we find (excluding Poppel's experiments) that of the 37 animals studied after castration a diminution in the gaseous metabolism was found in 31 cases, and no change observed in 6. Of the 19 patients, whose sexual glands had been removed or who were supposed to suffer from an insufficiency of the internal secretions of these glands, a diminution in the basal metabolism was found in 11 cases, and no change in the remaining 8.

Nitrogen Metabolism after Castration.

In animals nitrogen metabolism has been studied by several authors. Curatullo and Tarulli (1895) found no changes in the urinary nitrogen excretion of a bitch about 3 months after castration. In Poppel's experiments (1897), mentioned above, the nitrogen output was slightly increased.

Schultz and Falk (1899) give a few data concerning the nitrogen metabolism of 2 bitches a few days after castration, and of one of them 50 days after. The investigation performed twice on one bitch showed a slight increase in the nitrogen output; in the other a slight decrease occurred on the single occasion on which it was examined. It is unfortunate that two of these three experiments were performed so soon after castration.

Pinzani (1898) found in a bitch investigated 2 and 3 months after castration a diminution in the nitrogen and urea excreted.

Mossé and Oulié (1899) were unable to find any definite changes in the nitrogen excretion of a castrated bitch. It is not clear from their paper how soon after operation the experiment was performed.

Neumann and Vas (1902) found a negative nitrogen balance in one bitch 4 days after operation when the wound was suppurating.

In the above-mentioned dogs, Lüthje (1902) was unable to find any definite change in the nitrogen balance, although on autopsy of one of these dogs the nitrogen content of its body was found to have increased. He ascribed this to the larger size of the dog. Kalestinov (1913) studied the N-metabolism in one rabbit on a diet consisting of oats, hay, peas, starch and sugar. About 7 weeks after castration N-metabolism was found to be much decreased.

Heymans (1921) found a striking diminution in the nitrogen output of a capon during the first 7 days of starvation compared with the excretion of a normal cock under the same conditions. In 100 gm. of the capon's excreta an average of 5·2 gm. N was present, compared with 11·7 gm. N in that of the cock (about 56 per cent. less). Heymans ascribes this difference entirely to the different amount of fat stored in castrated and in normal animals. But this conclusion is not justified by other experiments by the same worker, in which he showed that during starvation the daily loss of weight of a capon is much less than that of a normal cock.

In the above-mentioned experiments of De Vita on 2 castrated puppies a decrease in the nitrogen metabolism was also found.

As far as I know there is only one investigation on the nitrogen metabolism of castrated human beings. The ovaries were removed from two women suffering from osteomalacia, in the hope of curing this disease. Zuntz (1913) determined the nitrogen balance in these two patients, but the observation was successfully completed in one woman only. It showed a definite decrease in the nitrogen metabolism 2 years after castration.

Summary.—Of the 11 animals whose nitrogen metabolism was studied with satisfactory technique,* a decrease of nitrogen was found in 6, increase in 1 and no change in 4 animals. In one woman with osteomalacia removal of the ovaries resulted in an increased nitrogen retention.

THE PRESENT INVESTIGATION.

The experiments described below were performed in the Laboratory of Experimental Pathology of the Medical Academy at Petrograd, from 1911 to 1917, jointly with Drs. Jebunev (1914), Kusnetzov (1914), Bogoslovsky (1921), Guinenevitch (1917), Merjeevsky (1916) and Rosnatovsky (1917). The detailed reports of all the experiments were published in Russian by my colleagues mentioned above. Now separated from them by a great distance I send them my greetings, and want once more to thank them heartily for their enthusiastic hard work in the Laboratory under my direction. A preliminary communication on a part of these experiments was published elsewhere (Korenchevsky, 1914).

Technique.

The experiments were performed on 11 dogs (4 males and 7 females) and 5 rabbits (4 males and 1 female). Before these animals were used for a metabolism experiment they were kept for 3 to 6 weeks in the laboratory. The dogs were put on a minimum ration, which was just sufficient to keep their

* Poppel's and Neumann and Vas's experiments are excluded.

weight constant. Once chosen, this ration remained the same both quantitatively and qualitatively for the rest of the dog's life in the laboratory. The dogs were fed on horseflesh and fat, and the rabbits on oats, cabbage and water. All the N-metabolism experiments on rabbits were performed on fasting animals, but in the greater number of the experiments on dogs these were fed. The metabolism was usually investigated over a period of 3 days at a time. Investigations on fasting animals started on the fourth day of fasting and lasted 3 days. It is well known that from the fourth to the eighth day of fasting the metabolism remains constant when calculated per kilo of body-weight. At the end of each fasting experiment the animal was fed abundantly until it regained its previous weight, when it was again put on the experimental ration. After castration the metabolism of the animal during starvation was studied under the same conditions as before the operation.

Nitrogen was estimated in food, urine and fæces by Kjeldahl's method. Fæces were not analysed in the fasting animals because of the very small amount of nitrogen and of the long time ensuing between each defæcation. Before each investigation was begun, as well as at the end, the animals were catheterized, and after catheterization their bladders were washed with warm saline solution in order to remove all urine from the bladder.

The gaseous metabolism was determined for periods of 23 hours. This method is similar to that used by Haldane, and can be described shortly as follows: The animal remained in an hermetically sealed, though at the same time well-ventilated apparatus. The expired H_2O and CO_2 were collected in a series of bottles containing sulphuric acid or potassium hydroxide. The retained oxygen was calculated by subtracting the figure representing the loss of weight of the animal (during the time it was in the apparatus) from the figure representing the total weight of all its excreta (CO_2, H_2O, urine and fæces) during the same period. The animals were weighed, catheterized, their temperature taken, and the bottles containing H_2SO_4 and KOH weighed on the expiration of the twenty-fourth hour of the experiment each day. For these latter weighings a large chemical balance was used sensitive to 0·03 gm. This balance was also used for weighing the apparatus containing the rabbit (but not that containing the dogs) while the animal remained in it (the in and out ventilating tubes were closed while the apparatus was weighed). The removal of the sexual glands was performed under aseptic conditions, and most of the wounds healed by first intention.

In nearly all cases the metabolism investigation was repeated to discover any variations in the results caused by the period of time after the operation. This period varied from 3 to 284 days in dogs and from 4 to 264 in rabbits after castration (see tables).

The absolute figures of metabolism and the calculations from them per 24 hours and kilo body-weight of the animals were given in the Russian papers, and in order to reduce the number of tables in this publication they are not repeated, but the results showing the *percentage* change from normal after castration are summarized in Tables I–IV. There is, however, one exception to this in the column showing the nitrogen balance. In this column the actual figures per kilo of body-weight and 24 hours are given to show the variation above (+)

TABLES I–IV.—THE METABOLISM OF ANIMALS AFTER CASTRATION COMPARED WITH THAT OF THE SAME ANIMALS UNDER THE SAME CONDITIONS BEFORE CASTRATION.*

TABLE I.—*Experiments on Dogs while Feeding. "Fat" Castrated Dogs with an Increase in their Weight of 5 per cent. and more after the Operation.*

No. of dog and sex.	Number of days after castration.	Average weight of dog per cent.	Diuresis per cent.	Nitrogen in urine per cent.	Nitrogen in fæces per cent.	Change in nitrogen balance in gm. per kilo and diem.	Gaseous metabolism. H_2O per cent.	Gaseous metabolism. CO_2 per cent.	Gaseous metabolism. O_2 per cent.
1. II. ♂	53	+ 21·1	− 4·3	−32·1	+48·0	+0·1317	−40·9	−20·1	−25·6
2. II. ♂	53	+ 10·0	− 4·8	−19·4	+40·0	+0·1509	+ 7·8	− 3·8	+ 3·3
9. II. ♂	55	+ 26·2	− 9·7	−28·8	+50·8	+0·0989	−25·1	−25·2	−24·1
5. I. ♀	60	+ 10·5	+16·0	−22·1	+ 8·9	+0·2377	+12·2	− 2·1	− 2·6
3*g*. I. ♂	72	+ 22·3	+32·1	−15·3	+ 4·8	+0·0347	−30·6	−18·6	−21·3
Average	59	+ 18·0	+ 5·9	−23·5	+30·5	+0·1308	−15·3	−14·0	−18·1
3*g*. II. ♂	98	+ 28·8	+18·2	−15·0	+51·7	+0·0391	−32·7	−42·0	−44·0
3*g*. III. ♂	149	+ 25·9	+ 8·5	− 9·1	+44·2	−0·0055	−12·7	−26·9	−22·9
Average	124	+ 27·3	+13·4	−12·0	+47·9	+0·0168	−22·7	−34·5	−[illegible]
12. I. ♀	174	+ 13·2	−10·7	−28·6	− 1·6	+0·1863	−40·7	−19·7	−18·1
12. II. ♀	206	+ 23·0	0	−32·11	−28·7	+0·1859	−41·62	−33·33	−17·64
12. III. ♀	234	+ 32·47	+ 5·0	−36·89	0	+0·1341	−46·6	−27·19	−26·7
9. III. ♂	228	+ 49·8	+15·4	−16·1	+42·2	−0·033	+18·5	−18·2	−14·7
Average	208	+ 29·6	+ 2·9	−28·4	+ 2·9	+0·1183	−27·6	−29·1	−19·3

* *Note.*—In this and the following tables the Roman numerals after the number of the animal denote the order of repetition of the metabolism estimation. The figures with + (increase) or − (decrease) denote the percentage changes from the normal in all columns except in that showing the nitrogen balance; in the latter actual figures in grammes are given.

TABLE II.—*Experiments on Dogs while Feeding. "Thin" Castrated Dogs with a Small Increase (below 5 per cent.) or Loss in their Weight after the Operation.*

No. of dog and sex.	Number of days after castration.	Average weight of dog per cent.	Diuresis per cent.	Nitrogen in urine per cent.	Nitrogen in fæces per cent.	Change in nitrogen balance in gm. per kilo and diem.	Gaseous metabolism. H_2O per cent.	Gaseous metabolism. CO_2 per cent.	Gaseous metabolism. O_2 per cent.
1. I. ♂	5	+ 3·7	+ 6·1	− 2·8	...	...	−23·1	− 0·9	−16·0
2. I. ♂	5	− 1·8	+30·0	+ 0·3	...	...	+ 2·9	+ 6·4	+ 8·8
9. I. ♂	3	+ 4·1	+ 2·0	−11·2	...	...	− 6·1	− 1·8	− 1·0
Average	4	+ 2·0	+12·7	− 4·6	...	...	− 8·7	+ 1·2	− 2·7
10. I. ♀	42	+ 3·5	−17·9	− 4·68	−38·6	−0·0557	+ 3·8	− 2·38	−10·42
10. II. ♀	58	+ 3·23	− 5·5	+ 2·34	−34·8	−0·0627	−14·28	+ 7·14	− 3·68
10. III. ♀	89	+ 2·71	+ 8·2	− 9·11	−10·9	+0·0326	− 4·76	− 5·35	−12·26
30. I. ♀	87	− 0·8	− 7·0	− 5·5	+ 4·2	+0·0016	+41·3	+17·5	+14·6
4. I. ♀	89	+ 2·8	+26·4	+ 0·03	+ 9·7	−0·0421	+29·0	+25·1	+32·4
13. I. ♀	41	− 2·71	−16·3	− 3·44	−38·4	+0·1110	+ 1·68	− 2·45	− 4·56
13. II. ♀	54	− 1·0	−29·4	−10·52	−20·5	+0·1283	− 2·52	− 6·37	− 7·61
13. III. ♀	72	+ 2·27	−24·1	−13·4	−54·1	+0·1680	− 0·84	− 7·35	− 8·62
Average	67	+ 1·25	− 8·2	− 5·54	−22·9	+0·0351	+ 6·67	+ 3·23	− 0·02
13. IV. ♀	103	− 5·2	−26·1	− 2·32	−35·6	+0·0952	+14·28	0	− 4·06
2. III. ♂	284	−10·2	−19·8	− 2·2	+ 7·3	+0·2260	+42·6	+20·9	+18·1
Average	194	− 7·7	−22·9	− 2·26	−14·2	+0·1596	+28·44	+10·4	+ 7·02

15. IV. ♀	.	193	.		.		.		.		.		.		.		.	
2. III. ♂	.	284	.	−10·2	.	−19·8	.	− 2·2	.	+ 7·3	.	+0·2260	.	+42·6	.	+20·9	.	+18·1
Average	.	194	.	− 7·7	.	−22·9	.	− 2·26	.	−14·2	.	+0·1596	.	+28·44	.	+10·4	.	+ 7·02

TABLE III.—*Experiments on Fasting Dogs.*

No. of dog and sex.	Number of days after castration.	Average weight of dog per cent.	Diuresis per cent.	Nitrogen in urine per cent.	Gaseous metabolism.		
					H_2O per cent.	CO_2 per cent.	O_2 per cent.
1. III. ♂	8	+ 9·6	−12·9	−27·7	− 1·3	− 2·8	+ 0·1
2. IV. ♂	8	− 1·2	+12·5	−11·9	+23·6	+40·1	+36·8
3. I. ♀	16	+11·8	+45·7	−19·8	− 6·2	− 2·3	− 8·3
Average	11	+ 6·7	+15·1	−19·8	+ 5·4	+11·7	+ 9·5
1. IV. ♂	68	+24·6	− 9·7	−42·4	−22·1	− 3·5	− 3·2
2. V. ♂	68	+12·1	−20·3	−46·5	+ 8·4	+12·8	+13·0
Average	68	+18·4	−15·0	−44·4	− 6·8	+ 4·6	+ 4·9
3. II. ♀	137	+15·9	+57·1	− 9·5	+50·8	+14·9	+30·9
1. V. ♂	171	+15·1	+17·7	−26·2	+19·5	− 2·9	− 0·8
2. VI. ♂	171	+12·6	− 6·2	−29·8	+29·4	− 3·1	+ 2·3
3. III. ♀	231	+22·5	−10·0	−75·9	+11·4	−14·5	−14·1
Average	191	+16·7	+ 0·5	−44·0	+20·1	− 6·8	− 4·2

TABLE IV.—*Experiments on Fasting Rabbits.*

No. of rabbit and sex.	Number of days after castration.	Average weight of rabbit per cent.	Diuresis per cent.	Nitrogen in urine per cent.	Gaseous metabolism		
					H_2O per cent.	CO_2 per cent.	O_2 per cent.
5. I. ♂	4	+ 1·1	−26·7	−30·6	+ 54·1	+ 7·1	+ 9·6
7. I. ♂	4	− 2·1	−40·0	−17·3	− 2·5	− 6·0	− 4·3
14. I. ♂	4	− 6·5	−23·1	−12·5	− 12·8	− 1·6	− 1·9
20. I. ♂	16	− 2·9	+ 8·3	− 7·3	+ 57·1	− 9·5	− 7·8
Average	7	− 2·6	−20·4	−16·9	+ 24·0	− 2·5	− 1·1
1. II. ♀	41	+ 6·6	...	...	+ 17·4	+ 0·5	+ 4·6
5. II. ♂	57	+ 1·1	−53·3	−55·7	+109·0	+ 9·7	+11·4
14. II. ♂	54	+ 2·4	−30·8	−49·5	− 7·1	− 9·4	− 6·2
20. II. ♂	60	− 0·3	−12·5	−38·2	+ 55·4	−39·5	−37·7
7. II. ♂	65	+ 6·0	−40·0	−41·5	+ 5·9	−14·0	−12·6
Average	55	+ 3·2	−34·1	−46·2	+ 36·1	−10·5	− 8·1
7. III. ♂	248	+24·8	−51·4	−52·6	− 9·5	−13·5	− 9·6
20. III. ♂	264	+30·9	−48·7	−41·4	+ 26·3	− 5·1	+ 7·0
Average	256	+27·9	−50·0	−47·0	+ 8·4	− 9·3	− 1·3

or below (—) normal. This gives a clearer idea of the amount of nitrogen retained or lost by the body than the percentage figures do, especially when the balances are very small.

All experiments on dogs while feeding were subdivided into two groups according to the change in their weight: (1) Dogs with an increase in their weight of 5 per cent. or more; and (2) dogs with a small increase (below 5 per cent.) or loss in their weight after operation. This subdivision is made because of the different changes in the metabolism, typical in most cases for "fat" and "thin" castrated dogs. The third group of experiments on fasting dogs and the fourth on fasting rabbits are separately tabulated.

Weight of Animals after Castration.

Taking into consideration all 11 dogs observed, a considerable increase (more than 5 per cent.) in weight is noticed in 7 of them, 4 males and 3 females, Nos. 1, 2, 3, 3*g*, 5, 9, 12 (see Tables I and III), and slight changes in weight above or below normal in 4 bitches (Table II). The weight of one dog, No. 2, remained increased up to 12·6 per cent. until the 171st day after castration (Table III), but had fallen by the 284th day to about 10·2 per cent. below normal (Table II). The small increase in the weight of dogs 1, 2 and 9 shown in Table II is explained by the short time which had elapsed since the operation (3–5 days). Later (Tables I and III) their weight increased. In nearly all cases the weight increases with the greater length of time after the operation (see for instance dogs Nos. 1, 3, 9 and 12). The weight of the rabbits did not show any considerable change during the first 2 months after castration, but was much increased 8 months later. The changes in metabolism may be demonstrated by a comparison of the amount of weight lost in one starvation experiment with that lost in another. In about half of the cases investigated this loss in weight was less pronounced after castration than it was before. In some animals this difference was very noticeable: *e.g.* about 10 months after castration rabbit No. 7 lost 6·1 per cent. of his previous weight during 3 days of starvation instead of 12·3 per cent. as before castration. These figures for rabbit No. 20 were 7·9 per cent. and 5·3 per cent. respectively.

Nitrogen Metabolism.

Nitrogen output in urine when investigated on fed dogs was quite definitely decreased in all the "fat" castrated dogs, sometimes in the first week after operation (Table II), and always later after about two months after operation. Repeated investigations showed a steadiness in this change of metabolism in all fat dogs except in the above-mentioned animal No. 2 at the time when it lost weight. In thin dogs with a small increase or even loss of weight this effect was less pronounced (dogs Nos. 10, 13, 30), or hardly any change was observed (dog No. 4). In some of the thin castrated dogs (in experiments 10, II, and 13, IV, Table II), which were observed over a considerable interval of time, and which at first showed a diminution in nitrogen excretion by the urine amounting to as much as 13·4 per cent., re-determination after a few weeks showed that the nitrogen excretion had increased to a nearly

normal amount. Decrease in nitrogen output by the urine when it occurred both in fat and in thin castrated animals was obvious not only when calculated per kilo of body-weight, but also in the total daily urinary nitrogen. This was noticed in all the experiments on fasting animals, and with one exception in all those on fed dogs.

If the amount of decrease in urinary nitrogen of fed dogs after castration is compared with that of similar fasting dogs a striking difference is seen; the decrease is much more pronounced in the latter case. This is especially clear when experiments on the same dogs while feeding and while fasting are compared (Table V).

TABLE V.—*Percentage change in Nitrogen Output in Urine of Dogs while Feeding and while Fasting compared with that of the same Animals under similar Conditions before Castration.*

Number of weeks after castration.	Dog No. 1.		Dog No. 2.	
	Feeding.	Fasting.	Feeding.	Fasting.
1–2	− 2·8	− 27·7	+ 0·3	− 11·9
9–10	− 32·1	− 42·4	− 19·4	− 46·5

It is an established fact that an animal during starvation oxidizes proteins more cautiously than fat or carbohydrate. Therefore the presence of a large amount of fat in the animal will decrease the protein oxidation. This fact might partly explain the different results obtained in the metabolism of fat dogs while fasting and while feeding after castration.

Table VI (see below), summarized from Tables I–III, corroborates this supposition from another point of view. But in some cases this explanation is not satisfactory. For instance, the experiments performed on dog No. 2 while feeding and fasting during the first two weeks following castration showed the same differences mentioned above (see Tables II and III), in spite of no change in weight occurring at this period. The absence of correlation in several cases between the degree of increase in weight and that of decrease in nitrogen metabolism is also another fact which cannot be explained in a satisfactory manner merely by the different amount of fat stored in castrated animals. For instance (in Table I), dog No. 9 greatly increased in weight in Experiment III, compared with Experiment II. In spite of that the decrease in urinary nitrogen was much greater during Experiment II. The same discrepancy is seen in Experiments 2, II, and 5, I, when compared with Experiments 3*g*, I–III (Table I); and in Experiments 2, V, and 2, VI, when compared with 3, II (Table III). Still more difficult to explain by the theory mentioned above is the striking decrease in the nitrogen metabolism in all four fasting rabbits whose weight during the first two months did not show any significant change (Table IV). It seems to me that this decrease in nitrogen metabolism can only be explained in a satisfactory manner by the supposition that after castration the amount of some principle or principles stimulating the oxidation of proteins is decreased.

Nitrogen excretion in fæces of the dogs was increased in 10, decreased in 7 and not changed in 4 experiments.

The absolute figures of the *balance of nitrogen metabolism* (Tables I and III) in almost all cases showed the same changes in protein oxidation as were shown by the excretion of urinary nitrogen. Only in a few experiments after castration was nitrogen retained in a lesser degree, when calculated per kilo of body-weight and compared with the normal, in spite of a decrease in the nitrogen output in the urine. Usually this could be explained by a larger increase in weight out of proportion to the small decrease in urinary nitrogen excreted. The nitrogen balance in the "thin" castrated dogs indicated a decrease of protein oxidation with the exception of dog No. 41 (Table II). Protein metabolism in dog No. 10 was finally lowered by the 89th day after castration.

Diuresis.

Diuresis did not show any constant or definite variation: in 17 experiments the excretion of urine was decreased, in 15 increased and in 1 there was no change.

Gaseous Metabolism.

Gaseous metabolism was studied simultaneously with that of nitrogen on the same dogs and rabbits, and upon female rabbit No. 1 in addition (Table IV). In the first week after castration the changes in the gaseous metabolism were variable and indefinite (Table II).

About two months later gaseous metabolism in "fat" castrated dogs was, in most cases, decreased, often to a considerable extent (Table I). However, amongst these dogs Nos. 2, II, and 5, I, showed little change in spite of a marked decrease in the nitrogen metabolism. Dog No. 2, III (Table II), which in a later period lost weight, even showed a considerable increase.

Amongst the "thin" castrated dogs, 2 (Nos. 4, I, and 30, I, Table II) had a markedly increased gaseous metabolism. Two others had a slightly decreased gaseous metabolism, although in a much lesser degree, than was found in "fat" castrated dogs.

As would be expected *à priori* (in feeding experiments), all castrated dogs which increased their weight considerably had a much lowered gaseous and protein metabolism. "Thin" castrated dogs, or those losing weight, showed comparatively small decrease, or even sometimes increase in their basal metabolism.

To what is the increased weight in "fat" castrated animals due? It is partly explained by lowered protein oxidation. This may sometimes be the whole explanation of the increase in weight, as is the case for instance with dog No. 5, I (Table I), in which an insignificant change in gaseous metabolism was combined with a great nitrogen retention. But in other dogs, especially those which greatly increased in weight, a diminished basal metabolism and concomitant low fat oxidation would appear to have been the chief reason for the increase in weight. This is quite clear in dogs with a comparatively small nitrogen retention (*e. g.* dog No. 3g, Table I). But, even in dogs with a large nitrogen retention, this would explain at the most one-third to one-half of the increase in weight (dog No. 12, Table I).

Increased deposition of fat in "fat" castrated animals was obvious at autopsy. It was also indicated by experiments on fasting animals after castration. As may be seen from Tables III and IV, the gaseous metabolism of the castrated animals was only exceptionally decreased (Rabbit No. 20, Table IV) compared with the animals while feeding. Usually if a relative decrease was found at all, it was small or irregular. In some instances a relative increase occurred. The following table, VI, combines data from Tables I and III comparing percentage change of the gaseous metabolism of "fat" castrated dogs while feeding, with that while fasting. From the table it may be seen:

(1) During feeding the gaseous metabolism of the castrated dogs was less than it was before castration.

(2) During fasting the gaseous metabolism was usually greater after castration than before.

(3) The relative effect of castration under the two conditions, feeding and fasting, was in the opposite direction.

TABLE VI.—*Gaseous Metabolism in "Fat" Castrated Dogs while Feeding and while Fasting Compared with that of the same Animals under Similar Conditions before Castration.*

Number of months after castration.	Percentage variation H_2O expired.		Percentage variation CO_2 expired.		Percentage variation O_2 consumed.	
	Feeding.	Fasting.	Feeding.	Fasting.	Feeding.	Fasting.
In first month	−8·7	+5·4	+1·2	+11·7	−2·7	+9·5
About 2 months	−15·3	−6·8	−14·0	+4·6	−18·1	+4·9
About 4–5 months	−22·7	+50·8	−34·5	+14·9	−33·5	+30·9
About 6–8 months	−27·6	+20·1	−29·1	−6·8	−19·3	−4·2

These facts, when taken into consideration with the variations in protein oxidation during fasting and feeding experiments (see p. 29), prove clearly the accumulation of fat in those castrated animals, whose weight increased after the operation. This accumulation of fat may partly explain the increased protein retention of castrated animals. The other cause of the lowered protein metabolism was indicated above (p. 29) namely, the absence in the castrated animal of some principle or principles, which stimulate protein metabolism in animals with normal sexual glands.

Why does not Castration always Produce the same Effect upon the Metabolism of the Animal? And why in Animals as well as in Human Beings do "Thin" and "Fat" Types of Castrati Exist?*

The causes suggested could be manifold: (1) After castration sexual activity decreases, and finally stops, but at different periods after castration, therefore less energy is expended. But this explanation does not suffice in

* In English there is not a general word describing a human being or animal deprived of its sexual glands. Following the usual medical habit of using Latin nomenclature, I suggest the use of *castratus* (sing.), *castrati* (plural).

many cases, *e.g.* for our animals which were all segregated. (2) The appearance of muscular and psychic laziness of varying degrees has been mentioned several times, but not as an obligatory result of the removal of the sexual glands. However, most animals kept in cages and kennels in the laboratory very soon become accustomed to their calm and confined life. Other animals, especially fox terrier dogs, remain restless, excitable, and are always noisy. In our experiments we did not use this kind of dog, and before the investigations began our animals had had plenty of time in which to accustom themselves to their new life. Although muscular and psychic laziness are generally considered the chief causes of obesity in castrated animals or human beings, it seems to me that the reverse might be more true—that the absence of stimulating hormones from the sexual glands occasions a lowered rate of metabolism, and that this is the cause of the observed laziness, not *vice versâ*. When once started, psycho-muscular laziness certainly lowers further the already low metabolism, each factor enhancing the other. (3) The most probable explanation of the different influences of castration upon the metabolism of animals may, in my opinion, be found in the anatomical and functional conditions of those other endocrine glands, which may replace or are antagonistic to the internal secretions of the sexual glands. It is quite obvious that the difference in the amount of hormones secreted by these glands might result in the prevailing influence of antagonistic or synergistic hormones.

Of glands of internal secretion the thyroid is most nearly related to the sexual glands. The atrophy of this organ after castration was observed in human beings by Tandler and Gross (1913), Falta (1913), and Baruch (1912), and in cattle and rabbits by Okunev in the laboratory under my direction.

Cecca and Okinchitz (1913) found much colloidal matter in the thyroid glands of castrated women, which also indicates a lowered activity of this organ. Conversely, thyroidectomy in young animals is followed by atrophy of the sexual glands (Hoffmeister, 1894; Biedl, 1916). The clinical observations of Veliaminov (1908, 1910), Baruch (1912), Lampé and Papusolu (1913), also indicate the very close interrelation of these glands. Mannaberg's (1913) observations are especially interesting, because by roentgenization of the ovaries he markedly lessened the symptoms of thyroid hypersecretion in 10 women suffering from Graves's disease.

The second gland in close relationship with the sexual glands is the hypophysis. Changes and hypertrophy were found after castration as well as after thyroidectomy (Rogowitsch, 1889; Tizzoni and Centani, 1890; Stieda, 1890; Fickera, 1905; Cimoroni, 1908; Biedl, 1916; Mottram and Cramer, 1923, and others), and atrophy of the sexual glands in hypopituitarism is well known (see Biedl).

The adrenals are the third group of glands considered to be obviously interrelated with the sexual glands. Feodossiev (1906), Shenck (1910) and Altenburger (1924) have shown that the suprarenal cortex hypertrophies after castration. When adrenals are given by the mouth, hypertrophy of the testis, and to a lesser degree of the ovaries, ensue (Hoskins and Hoskins, 1916). It is well known that there is a very great similarity between the cells of the suprarenal cortex and those of the corpus luteum. This suggested

to Mulon (1906), to conclude, that corpus luteum actually is "temporary suprarenal cortex."

Rebaudi (1908) and Kanevskaia (1919, 1923) found that, after castration, the size and amount of Langerhans islets in the pancreas are increased.

Unfortunately up to the present time no investigations have been made studying the changes in the glands mentioned above in "thin" and "fat" castrated animals. The only data given by the authors above mentioned state the difference in the degree of changes in the hypophysis, thyroid, pancreas and adrenal glands found after castration. All these glands influence metabolism. The thyroid gland increases it*; probably the hypophysis (Thompson and Johnston, 1905; Aschner and Porges, 1912; Benedict and Homans, 1912; Kestner, 1922, and Knipping, 1923) and adrenals (Aub, Forman and Bright, 1922), act in the same direction, although the data concerning the two latter glands are few and contradictory. The pancreas seems to decrease the nitrogen metabolism (MacLeod and Allen, 1923).

When all these facts are considered, it seems very probable that the decrease in metabolism after removal of the sexual glands is partly of a secondary nature, and due to the atrophy of the thyroid and hypertrophy of the islets of Langerhans. Their influence is counteracted by hypertrophy of the suprarenal cortex and hypophysis.

CONCLUSIONS.

(1) In most cases removal or hyperfunction of the sexual glands in animals as well as in human beings is followed by an increase in weight and an accumulation of fat ("fat" castratus or eunuch).

(2) However, in approximately 40 per cent. of cases, castration or hypofunction of the gonads and ovaries do not produce this effect ("thin" castratus or eunuch).

(3) Our experiments accordingly showed that both nitrogenous and, in most cases, gaseous metabolism were decreased after castration in "fat" castrated dogs.

(4) In "thin" castrated dogs this fall in the nitrogenous and gaseous metabolism was usually less pronounced or absent; moreover, the gaseous metabolism was sometimes considerably increased.

(5) Nitrogen metabolism of fasting dogs and rabbits was much lowered after castration in all the cases investigated, but the gaseous metabolism did not change at all or was only a little decreased, or was even increased.

(6) The fall after castration in the nitrogen metabolism of a fasting animal when compared with that of the same animal while feeding is always much greater; the converse was noticed in the gaseous metabolism.

(7) All these facts mentioned above show that not only an accumulation of fat but also a retention of protein in the body are the causes of the increased weight of "fat" castrati or eunuchs.

(8) The experimental results obtained in this investigation, and the other experimental and clinical data, concerning the internal secretion of the sexual

* See literature in Biedl (1916) and Korenchevsky (1914).

glands, suggest the presence of specific hormones in these glands, which influence the body-metabolism.

(9) The question whether the sexual hormones influence metabolism, directly or indirectly, through the other glands of internal secretion cannot be definitely stated at the present time.

(10) As an explanation of the different results of castration upon metabolism the following hypothesis is suggested: these results are due not only to the direct influence upon metabolism of the removal of the sexual glands, but also to the secondary changes produced by castration in the thyroid, hypophysis, adrenals and pancreas, as well as to the varying degree of functional efficiency of these glands before castration.

I wish to express my sincere gratitude to Professor C. J. Martin for his criticism of this paper.

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ON STRUCTURES WHICH DEVELOP IN CERTAIN CULTURE MEDIA AND RESEMBLE COLONIES OF MICRO-ORGANISMS.

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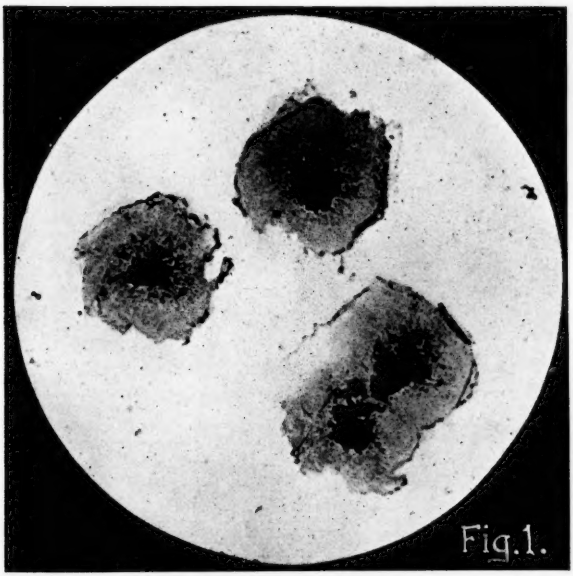
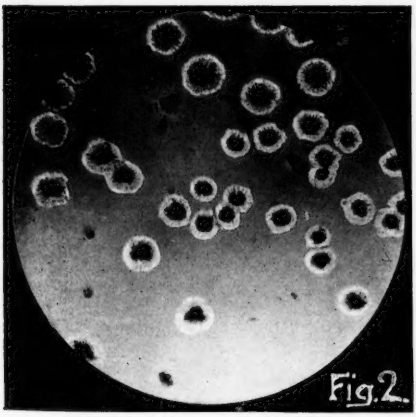
In the course of a long series of experiments on the cultivation of filter-passing viruses, a peculiar phenomenon was encountered which caused much trouble until the proper explanation was found. In brief, the phenomenon was the formation of artificial colonies on the surface and in the depths of certain culture media. The colonies were believed for some time to be aggregates of very minute organisms, because the colonies definitely grew, could be propagated from tube to tube, and could be maintained in subculture, apparently indefinitely, when the proper medium was employed. It is now certain that these apparent colonies are artefacts, and that they are to be explained along the same lines as those of F. W. Twort and D. N. Twort (1921), who encountered a similar phenomenon when they were attempting to cultivate filter-passing organisms. The explanation given by these authors for their artificial colonies cannot hold in detail for these under consideration now, and the chief reason for the publication of these notes is to draw attention to a phenomenon which is very liable to mislead other workers who expect to find small colonies of very minute organisms.

From analogy with the results obtained in cultures of the organism of pleuro-pneumonia of cattle, it was hoped that it would prove possible to secure visible colonies of other organisms which were themselves so minute as to be almost invisible under the microscope. To explore this possible line of investigation many attempts were made to cultivate various viruses, on the surface and in the depths, of nutrient agar media of diverse composition, enriched with serum, tissue, or both serum and tissue. A smaller number were also carried out using whole plasma as the culture medium. In a fair proportion of the experiments with media enriched as mentioned above small points appeared in the inoculated tubes, and only along the line of inoculation, after two or three days' incubation at body temperature. As time passed these grew and developed into small, flat, circular masses in the case of the surface growths, and spherical masses in the shake cultures. When the masses were crowded together they remained very small, but if they were separate a single colony might grow to $\frac{1}{2}$ mm. in diameter.

Fig. 2 shows the appearance of some of these surface colonies photographed through a 1-in. objective with oblique illumination. They are circular in outline, with a somewhat shiny surface and a darker centre. The centre may appear to project slightly under some illuminations and to be depressed with





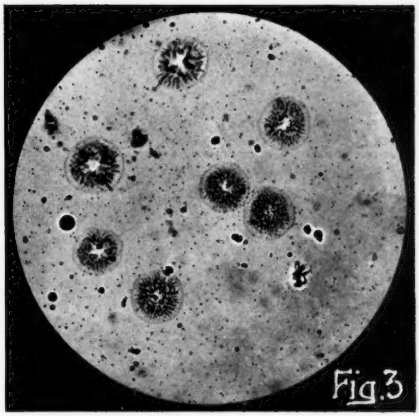


Fig. 1.—Pseudo-colonies. Impression preparation on cover-glass. Giemsa's stain. × 960 approx.
Fig. 2.—Pseudo-colonies. Serum agar slope. Oblique illumination. × 36 approx.
Fig. 3.—Pseudo-colonies. Transmitted light. × 36 approx.

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others. The colonies can be broken up with a platinum loop and detached fairly readily.

Fig. 3 shows the appearance under transmitted light with the same magnification. Here, in addition to the circular outline, the most noteworthy feature is the hair-ball structure of the mass. The lines suggesting hairs are very variable in different specimens and are often more prominent than in the figure; on the other hand, they may be faint, and the whole colony seem very transparent.

Impression preparations may be made on cover-glasses, and in these whole colonies may become detached and after fixation can be stained. Fig. 1 shows the result after intensive staining with Giemsa's stain. The central mass resembles a dense clump of tiny organisms, and the surrounding thinner parts show smaller aggregations of very minute particles. The whole appears to be held together by a structureless matrix.

Implantations from these colonies could be subcultivated apparently indefinitely. If a thick planting was carried out on to a new tube a film grew; if a dilution was planted separate colonies developed. On the whole, the appearance developed best and more rapidly in an anaërobic jar, but the same result was secured under aërobic conditions.

Emulsions of the colonies had no effect on animals, and fundamentally the same type of colony was secured from several viruses of quite different natures, and later a similar appearance developed on a slope inoculated from a sterile Noguchi tube. Doubts as to the nature of the colony arose, and, to make a long story short, it was found that an emulsion of the colonies could be boiled for five minutes or autoclaved for half an hour and give rise to as good a growth on subculture as an untreated sample kept as a control. This seems to prove that the "colonies" are inanimate, and it was thought to be worth while to try and determine their true nature.

The clue to the explanation of the artefact was obtained by examining whole "colonies," cut out from the medium, under the high power of the microscope, and determining how impression preparations of the colonies behaved to chemical reagents.

The ideal colony appeared to consist of a structureless matrix, thick in the centre and very thin at the periphery, in which there were embedded roughly spherical masses of varying size. As a rule, there was one larger mass in the centre. This central mass, when of good size (15 to 20 μ), was found to have a faintly ragged outline and radiating lines from its centre: in short, it resembled a sphærocrystal. The masses were insoluble in water, alcohol, chloroform, ether and other organic solvents. They were completely altered by 1 per cent. hydrochloric acid in alcohol, and appeared to be broken up and partly dissolved. Treatment by chloroform, after acid alcohol, appeared to dissolve still more, but the matrix remained and the cast of the semi-crystalline mass persisted, so that it was difficult to be sure how far the mass had dissolved out and how far it was merely altered by the reagents. The dispersed colonies in serum, agar, shake cultures and in whole plasma, when cut out and examined under the microscope, proved to be composed also of sphærocrystals in masses. The amount of material from colonies on media was too small for any serious chemical examination.

The fact that serum or tissue appeared to be requisite for the formation and propagation of the colonies suggested that these substances were the source of supply of the constituents of the colony. When serum is seeded with a suspension of the colonies and incubated, a very fine sandy deposit forms. This deposit, when examined under a $\frac{1}{12}$-in. objective, is seen to consist of well-formed sphærocrystalline masses very similar to, but much better formed and cleaner looking than, the sphærocrystalline masses in the colonies described above.

A bottle containing 500 c.c. of fresh sterile serum was therefore seeded with emulsion of the artificial colonies and incubated for some days. The fine sandy deposit was collected on filter-paper and well washed with water. The deposit was suspended in water, a drop or two of strong hydrochloric acid added, then one volume of ether, and the whole shaken vigorously. The particles broke up and dissolved almost completely, either in the ether layer or the watery one. The ether layer gave a greasy residue on evaporation, which was acidic and soluble in caustic alkali to give a soapy solution. The amount was small, but there can be no doubt that fatty acids accounted for the bulk of the ether-soluble material. The water layer gave on evaporation a small residue which contained calcium, magnesium and a little phosphate.

It is believed therefore that the sphærocrystalline masses formed in serum, when incubated after inoculation with the artificial colonies, consist for the most part of calcium and magnesium soaps; and further, that the artefact, believed at one time to be a colony of organisms, is also due essentially to a crystallization of calcium and magnesium salts of fatty acids. Both these soaps may be expected to be formed under the conditions of our cultivation experiments, and they will remain dispersed in a complex colloidal system such as nutrient serum agar; if, however, a focus of crystallization be started by inoculating a few small crystals, those crystals will grow and may form the nucleus of a colony.

This explanation will account for the bulk of the colony, but it leaves the matrix unexplained. Possibly this is some other constituent of the medium altered by the separation of the soaps.

This explanation will also account for the fact that the artificial colony may occasionally develop on incubation of uninoculated serum agar slopes, though this is quite unusual. The artefact may also be initiated by scratching the surface of serum agar slopes with a freshly flamed platinum loop or other manipulation, and incubating the tube for some days.

It may be noted in passing that once these artefacts have developed the medium is of no value for the cultivation of the organism of pleuro-pneumonia of cattle. This observation may be correlated with the well-known deleterious action of soaps in culture media, and explain why fresh serum is preferable to old for enriching culture media.

It will be remembered that F. W. Twort and D. N. Twort described the formation of small artefacts behaving similarly to those described above when they were endeavouring to grow saprophytic filter-passing organisms. For that purpose they employed media containing small amounts of sulphides, or incubated the tubes in an atmosphere containing traces of sulphuretted hydrogen. They give reasons for supposing that their colonies were growing

aggregates of sulphur crystals. The medium used in the present experiments contained no sulphides, and all sulphuretted hydrogen was carefully removed from the hydrogen employed in the anaërobic experiments; separation of sulphur is therefore unlikely, and is excluded by the behaviour of the colony towards organic solvents. The phenomenon, however, is fundamentally similar to that described by F. W. Twort and D. N. Twort, in that a crystallization process can mimic colony formation very closely.

SUMMARY.

(1) An artificial colony similar to that previously recorded by F. W. Twort and D. N. Twort is described.

(2) The artefact is due to the separation of calcium and magnesium soaps out of the culture medium.

REFERENCE.

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CULTURES FROM SINGLE CELLS.

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THE method devised by Topley, Barnard and Wilson (1921–22) has proved of value both for growing bacterial colonies from single organisms and for tissue culture (Drew, 1923).

It consists essentially in making a suitable preparation on a glass slide and using a cover-glass of quartz instead of an ordinary glass one. The organism or cell is then covered with a minute globule of mercury, and the whole exposed to a suitable bactericidal light source. The result is that all the cells are killed except that protected by the mercury globule, and by a simple technique a growth can be obtained from this cell. Perhaps the greatest practical objection to the method is that a supply of quartz cover-glasses is needed, and that they are of necessity expensive. They must be cut from either crystalline or fused silica, usually the former, and optically worked to the required thickness. There is no necessity to use cover-glasses of such accuracy of surface, but they can hardly be made in any other way.

It has recently been observed at the National Institute for Medical Research, in the course of some experiments having an entirely different object, that selenite in thin plates is sufficiently transparent to the ultra-violet to use in place of quartz for this purpose. Selenite is hydrated sulphate of lime ($CaSO_4$, $2H_2O$), and occurs naturally in large transparent crystals which have perfect cleavage planes. From such crystal masses films of even thickness and perfect optical surface may readily be obtained. It splits in the same way as mica and has a similar appearance, but is far more transparent to ultra-violet radiations. A convenient tool with which to split up a large piece is an old safety-razor blade. This can be introduced into the edge of the mass, and by a gentle levering action a thin plate is separated. The substance is not hard like quartz, although its optical polish is of a high order. It is therefore best to avoid any cleaning or rubbing of the surface, and not to attempt to use a cover-glass more than once. Its relative cheapness renders such a procedure unnecessary. Its optical properties do not differ sufficiently from glass or quartz to preclude its use for the purpose now described, but it is not suitable in cases where the utmost microscopical resolution is necessary.

The exposure to a light source such as a mercury-vapour lamp is not appreciably increased as the result of substituting thin selenite films for quartz.

If it is necessary to heat selenite it should not be raised to a high temperature, as it speedily loses its water of crystallization and becomes opaque. It can be rapidly passed through a flame, but it is preferable to put the prepared cover-glasses into a Petri dish and sterilize the whole in a hot-air sterilizer.

Cover-glasses of selenite in any required thickness or size can be obtained from Messrs. Hawksley & Sons, 83, Wigmore Street, W.

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